REMARKS/ARGUMENTS

Upon entry of this reply, claims 1, 3, 14-25, 27, 38, and 42 will be pending in the application. Claims 2, 4-13, 26, 28-37, and 49-41 are canceled. No new matter is introduced by way of reply. Applicants note with appreciation withdrawal of the prior objection and rejections in view of Applicants' prior amendments and remarks.

I. Claims 1, 3, 15, 27, 38, and 42 are patentable over Nicolaides 1.

Claims 1, 3, 15, 27, 38, and 42 are rejected under 35 U.S.C. § 103 for alleged obviousness over Nicolaides 1 (United States Published Application 2002/0068284). Applicants traverse the rejection.

To establish a *prima facie* case of obviousness, three requirements must be satisfied: first, there must be some suggestion or motivation to modify the reference or to combine the reference teachings; second, there must be a reasonable expectation of success for achieving the claimed invention and its particular results; and, third, the prior art references must teach or suggest all the claim limitations. *See In re Vaeck*, 20 U.S.P.Q. 2d 1438 (Fed. Cir. 1991).

Claims 1, 3, and 15 recite methods for generating antibiotic resistant bacteria by introducing a dominant negative allele of a mismatch repair gene into the bacteria which becomes hypermutable as a result; contacting the bacteria with a plurality of antibiotics; selecting bacteria resistant to the antibiotics; and culturing the selected bacteria. Multi-antibiotic resistant bacteria having a dominant negative allele of a mismatch repair gene are encompassed by claims 38 and 42.

The Office action alleges that Nicolaides 1 indicates that the hypermutable bacteria taught therein may have one or multiple mutations and traits. (Office action at page 4.) Nicolaides 1 teaches that cells expressing dominant negative mismatch repair (MMR) genes have altered mismatch control pathways, thereby altering a gene or set of genes controlling a *single* phenotype. (Nicolaides 1, [0058].) In other words, Nicolaides 1 teaches that the hypermutable bacteria described therein may have one or more mutations resulting in any *one* of a number of altered phenotypes, such as resistance to an antibiotic (*e.g.*, kanamycin) (Nicolaides 1, [0058]), heat resistance, or high recombinant protein production (Nicolaides 1, Example 3). Nicolaides 1 does not teach or suggest that the one or more mutations resulting

from expression of the dominant negative allele of the MMR gene yields more than one altered bacterial traits. The skilled artisan would have no reasonable expectation of success in generating bacteria having multiple new traits, such as resistance to more than one antibiotic. Accordingly, claims 1, 3, 15, 38, and 42 are patentable over Nicolaides 1. Reconsideration and withdrawal of the rejection is respectfully requested.

II. Claims 1, 19, 27, and 38 are patentable over Iris in view of Stemmer, Johnston, Aronshtam, LeClerc, Drummond, Moreland, and Morris.

Claims 1, 19, 27, and 38 are rejected under 35 U.S.C. § 103 for alleged obviousness over U.S. Patent No. 6,221,585 to Iris *et al.* ("Iris") in view of U.S. Published Application 2002/0049104 to Stemmer *et al.* ("Stemmer"), U.S. Patent No. 6,043,048 to Johnston *et al.* ("Johnston"), Aronshtam and Marinus (*Nuc. Acids Res.*, 24(13):2498-2504 (1996)) ("Aronshtam"), LeClerc *et al.* (*Science*, 274:1208-1211 (1996)) ("LeClerc"), Drummond *et al.* (*J. Biol. Chem.*, 271(33):19645-19648 (1996)) ("Drummond"), Moreland *et al.* (*Cancer Res.*, 59:2102-2104 (1999)) ("Moreland"), and Morris *et al.* (*J. Infect. Dis.*, 171:954-960 (1995)) ("Morris"). Applicants disagree with the rejection.

The Iris reference is cited for its alleged teaching that it is beneficial to select cells having a phenotype of interest, such as antibiotic resistance. (Office Action dated February 23, 2003, at page 8.) Stemmer is relied upon for its alleged teaching of the use of mismatch repair deficiency to induce phenotypic variation. (Office Action dated October 20, 2003, at page 6.) The Aronshtam reference is cited for its alleged teaching of the use of dominant negative alleles of mismatch repair genes to increase mutagenicity in bacteria. (Office Action dated October 20, 2003, at page 7.) The Johnston reference is cited for its purported demonstration that it is known in the art to identify antibiotic-resistant bacteria by culturing cells in the presence of the antibiotic against which resistance is sought. (Office Action dated February 23, 2003, at page 8.) The LeClerc, Drummond, and Moreland references are relied upon for their alleged teachings that mutations in mismatch repair genes result in generation of drug resistance. (Office Action dated October 20, 2003 at page 7.) Morris is cited to allegedly provide motivation to generate and study bacterial cells having resistance to multiple antibiotics. (Office Action dated October 20, 2003 at page 7.) Even assuming that the Examiner's characterization of the teachings of each of the cited references is correct,

which Applicants deny, Applicants assert that the Examiner is employing impermissible hindsight analysis to pick and choose among elements of the prior art to arrive at the present invention. As case law mandates, "[i]t is impermissible within the framework of Section 103 to pick and choose from any one reference only so much of it as will support a given position, to the exclusion of other parts necessary to the full appreciation of what such reference fairly suggests to one of ordinary skill in the art." *In re Wesslau*, 147 U.S.P.Q. 391, 393 (C.C.P.A. 1965).

The Federal Circuit has stated in no uncertain terms that the importance of the requirement of a motivation to combine prior art references in asserting an obviousness rejection cannot be overlooked:

> When patentability turns on the question of obviousness, the search for and analysis of the prior art includes evidence relevant to the finding of whether there is a teaching, motivation, or suggestion to select and combine the references relied on as evidence of obviousness.... "The factual inquiry whether to combine references must be thorough and searching." It must be based on objective evidence of record. This precedent has been reinforced in myriad decisions, and cannot be dispensed with.

In re Lee, 277 F.3d 1338 (Fed. Cir. 2002) (citations omitted).

The Examiner asserts that the requisite motivation to combine the Iris and Stemmer references derives from the general aim of each of the references to identify genetic sources of phenotypes. This, however, is the end of any similarity between the two references. The Stemmer and Iris references propose two wholly different methods for identifying genes associated with a phenotype. The methods of the Stemmer reference rely on generation of genetic diversity of chimeric nucleotide sequences. In the Stemmer method, members of a library of diverse conjoint polynucleotides are introduced into a host cell for expression and selection. (Stemmer, [0054].) Vectors conferring a desired phenotype are recovered and subjected to diversification until an optimized set of elements are identified. (Stemmer, Figures 3 and 4.) Thus, the method of Stemmer begins with a nucleic acid molecule having an unknown function and derives the phenotype associated therewith. In contrast, the Iris reference first identifies a phenotype of interest and then compares a homogeneous population of nucleic acid molecules from a population having the phenotype of interest to a second homogeneous population of nucleic acid molecules not having the phenotype of

interest. (Iris, Col. 8.) Mismatched duplex nucleic acid molecules between the first and second populations purportedly contain the genes that confer the phenotype of interest. In short, the methods of Iris and Stemmer are completely polar approaches to identifying genes associated with a phenotype. The ordinarily skilled artisan thus would not have been motivated to combine the teachings of the two references.

Additionally, the Stemmer and Iris references fail to teach or suggest generation of a hypermutable organism by introducing a dominant negative allele of a mismatch repair gene. The Iris reference nowhere mentions how the organism exhibiting the phenotype of interest– antibiotic resistance - is generated. Stemmer suggests diversification of conjoint constructs by employing site-directed mutagenesis in mismatch repair-deficient host organisms. (Stemmer, [0113] and [0119].) However, contrary to the Examiner's assertion that Stemmer teaches that defective mismatch repair can induce phenotypic variation, the use of mismatch repair deficiency to generate mutations within an organism is nowhere taught or even suggested by Stemmer. Stemmer simply employs mismatch repair-deficient cells in order to prevent correction of the mutations introduced by site-directed mutagenesis. Indeed, Stemmer teaches away from approaches wherein the entire genetic background is the subject of selection because "deleterious effects often counterbalance the desirable effects, reducing the overall success and efficiency of the program." (Stemmer, [0080].) Stemmer thus manipulates genetic elements in a synchronized manner to exert control over a phenotype. (Stemmer, [0084].) The Aronshtam reference, however, describes increased *spontaneous* mutagenicity in cells comprising dominant negative mutations in a mismatch repair gene. (Aronshtam, abstract.) The spontaneous mutagenicity associated with dominant negative mutations of mismatch repair genes induces random mutations throughout the host's genome. One having ordinary skill in the art thus would not be motivated to combine the Stemmer disclosure with the teaching of Aronshtam. Likewise, the ordinarily skilled artisan would not be motivated to combine LeClerc, Drummond, or Moreland with Stemmer. In addition, the LeClerc reference identifies hypermutable strains of bacteria having defective mismatch repair; however, the mutations are not dominant negative, as they can be complemented by wild-type genes. (LeClerc, Table 3.) The Drummond and Moreland references link antitumor drug resistance to mismatch repair deficiency in ovarian cancer cells. One of ordinary skill in the art, however, would not be motivated to refer to Moreland or Drummond

to develop methods for generating antibiotic resistance in bacteria. The Morris reference links multidrug resistance in *M. tuberculosis* to an accumulation of mutations in genes encoding drug targets but teaches or suggests nothing regarding mismatch repair deficiency or generation of antibiotic-resistant bacteria using a dominant negative allele of a mismatch repair gene. One of ordinary skill in the art would not look to the Morris reference in seeking to develop methods for generating antibiotic-resistant bacteria. The relationship of the Morris reference to the present invention is tenuous at best.

Moreover, as acknowledged by the Examiner, the Iris, Stemmer, Johnston, and Aronshtam references fail to teach or suggest introduction of a dominant negative allele of a mismatch repair gene to yield multiple antibiotic resistance as presently claimed. LeClerc, Drummond, Moreland, and Morris fail to remedy this deficiency. Although LeClerc suggests a link between the mutator phenotype and drug resistance, that reference does not teach or suggest introduction of a dominant negative allele of a mismatch repair gene into bacteria to generate antibiotic-resistant microbes. The Drummond, Moreland, and Morris references also fail to teach or suggest introduction of a dominant negative allele of a mismatch repair gene into bacteria to generate antibiotic-resistance.

Applicants further assert that, even assuming that the requisite motivation to combine the selected elements of the cited references exists, which Applicants deny, the ordinarily skilled artisan would have no reasonable expectation of success in arriving at the presently claimed methods and bacteria. At best, the Examiner's reasoning amounts only to an obviousness to try rationale; "obvious to try," however, is not the proper legal standard under 35 U.S.C. § 103. *In re O'Farrell*, 853 F.2d 894, 903 (Fed. Cir. 1988).

The Examiner asserts that the Johnston reference suggests that multiantibiotic resistant bacteria can be generated by culturing cells in a medium containing two or more antibiotics. Applicants disagree with this characterization of the Johnston reference. Johnston describes the very limited circumstance of multiantibiotic resistance wherein bacterial beta-lactamases are induced by a beta-lactam antibiotic and subsequently challenged with an indicator antibiotic, thereby preventing false susceptibility results to the indicator antibiotic. Culturing of the bacterial cells in the indicator antibiotic does not induce bacterial resistance thereto. Thus, it would not be obvious to one of ordinary skill in the art to generate

multiantibiotic-resistant bacteria simply by culturing the bacteria in medium containing two or more antibiotics.

Applicants request that the Examiner support the assertion that one of ordinary skill in the art would be motivated to re-stabilize the hypermutable bacterial cells once cells having the phenotype of interest had been isolated. (Office action dated October 20, 2003 at page 9.) 37 C.F.R. § 1.104(d). Absent such support, Applicants maintain that it would not have been obvious to one of ordinary skill in the art to make the antibiotic resistant bacteria genetically stable. Deficiencies of the cited references cannot be remedied by general conclusions about what is "basic knowledge" or "common sense." *In re Lee*, 277 F.3d at 1344-45. Common knowledge and common sense do not substitute for authority when the law requires authority. *Id.*

In short, Applicants assert that not one of the requisite elements of a *prima facie* case of obviousness of claims 1, 19, 27, and 38 has been established on this record. Reconsideration and withdrawal of the rejection is respectfully requested.

III. Claims 1, 3, 27, 38, and 42 are patentable over Iris in view of Stemmer, Johnston, and either of Nicolaides 2 or Nicolaides 3, further in view of LeClerc, Drummond, Moreland, and Morris.

Claims 1, 3, 27, 38, and 42 are rejected for alleged obviousness over Iris in view of Stemmer, Johnston, and either of Nicolaides 2 (Nicolaides *et al.*, *Mol. Cell. Biol.*, 18(3):1635-1641 (1998)) or Nicolaides 3 (U.S. Patent No., 6,146,894), further in view of LeClerc, Drummond, Moreland, and Morris. Applicants traverse the rejection.

The above remarks regarding the lack of *prima facie* obviousness of claims 1, 19, 27, and 38 in view of the Iris, Stemmer, Johnston, LeClerc, Drummond, Moreland, and Morris references are equally applicable to the present rejection and are incorporated here by reference to Part II.

As previously explained, the Stemmer, Iris, and Johnston references fail to teach or suggest generation of a hypermutable organism by introducing a dominant negative allele of a mismatch repair gene. Nicolaides 2 or Nicolaides 3 cannot be relied upon to remedy this deficiency. Stemmer suggests diversification of conjoint constructs by employing site-directed mutagenesis in mismatch repair-deficient host organisms. (Stemmer, [0113] and

[0119].) However, the use of mismatch repair deficiency to generate mutations within an organism is nowhere taught or even suggested by Stemmer. Stemmer simply employs mismatch repair-deficient cells in order to prevent correction of the mutations introduced by site-directed mutagenesis. Indeed, Stemmer teaches away from approaches wherein the entire genetic background is the subject of selection because "deleterious effects often counterbalance the desirable effects, reducing the overall success and efficiency of the program." (Stemmer, [0080].) Stemmer thus manipulates genetic elements in a synchronized manner to exert control over a phenotype. (Stemmer, [0084].)

Nicolaides 2 and Nicolaides 3, however, describe increased *spontaneous* mutagenicity in cells comprising a dominant negative allele of a mismatch repair gene. (Nicolaides 3, Col. 3.) The spontaneous mutagenicity associated with dominant negative mutations of mismatch repair genes induces random mutations throughout the host's genome. One having ordinary skill in the art thus would not be motivated to combine the Stemmer disclosure with the teaching of either of Nicolaides 2 or Nicolaides 3. Likewise, the ordinarily skilled artisan would not be motivated to combine LeClerc, Drummond, or Moreland with Stemmer.

In short, Applicants assert that a *prima facie* case of obviousness of claims 1, 3, 27, 38, and 42 has not been established on this record. Reconsideration and withdrawal of the rejection is respectfully requested.

IV. Claims 1, 3, 14-25, 27, 38, and 42 are patentable over either of (A) Iris, Stemmer, Johnston, and Aronshtam or (B) Iris, Stemmer, Johnston, either of Nicolaides 2 or 3, LeClerc, Drummond, Moreland, Morris, Lin, Chang, Setterstrom, and The Merck Index.

Claims 1, 3, 14-25, 27, 38, and 42 are rejected for alleged obviousness over either of (A) Iris, Stemmer, Johnston, and Aronshtam or (B) Iris, Stemmer, Johnston, either of Nicolaides 2 or 3, LeClerc, Drummond, Moreland, Morris, U.S. Patent No. 6,025,400 to Lin ("Lin"), U.S. Patent No. 6,043,220 to Chang *et al.* ("Chang"), U.S. Patent No. 6,410,056 to Setterstrom *et al.* ("Setterstrom"), and The Merck Index (1983, pages 2036, 5032-5033, and 6448-6449). Applicants disagree with the rejection.

The above remarks regarding the lack of *prima facie* obviousness of claims 1, 19, 27, and 38 in view of the Iris, Stemmer, Aronshtam, Johnston, LeClerc, Drummond, Moreland,

and Morris references are equally applicable to the present rejection and are incorporated here by reference to Part II. Additionally, the above remarks regarding the lack of *prima facie* obviousness of claims 1, 3, 27, 38, and 42 in view of the Iris, Stemmer, Johnston, Nicolaides 2 or Nicolaides 3, LeClerc, Drummond, Moreland, and Morris references are equally applicable to the present rejection and are incorporated here by reference to Part III.

Furthermore, bacterial resistance to a plurality of antibiotics including those listed in claims 14-25 has not been established. The Lin, Chang, and Setterstrom references and The Merck Index are relied upon by the Examiner to show that the antibiotics of claims 14-25 were known in the art. However, no motivation to combine those references with any of Iris, Stemmer, Aronshtam, Johnston, Nicolaides 2 or Nicolaides 3, LeClerc, Drummond, Moreland, and Morris or a reasonable expectation of success in generating resistance to the antibiotics identified by introducing a dominant negative allele of a mismatch repair gene into bacteria has been established on the present record. Absent these elements, a *prima facie* case of obviousness of claims 1, 3, 14-25, 27, 38, and 42 cannot be demonstrated. Reconsideration and withdrawal of the rejection is respectfully requested.

CONCLUSION

In view of the foregoing, Applicants believe all claims now pending in this application are in condition for allowance. The issuance of a Notice of Allowance at an early date is respectfully requested.

If the Examiner believes a telephone conference would expedite prosecution of this application, the undersigned may be contacted at 215-557-5908.

Respectfully submitted,

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